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Transfer of Hepatitis B Virus Genome by Adenovirus Vectors into Cultured Cells and Mice: Crossing the Species Barrier

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For the study of hepatitis B virus infection, no permissive cell line or small animal is available. Stably transfected cell lines and transgenic mice which contain hepadnavirus genomes produce virus, but—unlike in natural infection—from an integrated viral transcription template. To transfer hepadnavirus genomes across the species barrier, we developed adenovirus vectors in which 1.3-fold-overlength human and duck hepatitis B virus genomes were inserted. The adenovirus-mediated genome transfer efficiently initiated hepadnavirus replication from an extrachromosomal template in established cell lines, in primary hepatocytes from various species, and in the livers of mice. Following the transfer, hepatitis B virus proteins, genomic RNA, and all replicative DNA intermediates were detected. Detection of covalently closed circular DNA in hepatoma cell lines and in primary hepatocytes indicated that an intracellular replication cycle independent from the transferred linear viral genome was established. High-titer hepatitis B virions were released into the culture medium of hepatoma cells and the various primary hepatocytes. In addition, infectious virions were secreted into the sera of mice. In conclusion, adenovirus-mediated genome transfer initiated efficient hepatitis B virus replication in cultured liver cells and in the experimental animals from an extrachromosomal template. This will allow development of small-animal systems of hepatitis B virus infection and will facilitate study of pathogenicity of wild-type and mutant viruses as well as of virus-host interaction and new therapeutic approaches.

Chronic hepatitis B is one of the most common and severe viral infections of humans worldwide. Currently, 5% of the world's population are persistently infected with hepatitis B virus (HBV) (57). Infected individuals are at high risk of developing liver cirrhosis and, eventually, hepatocellular carcinoma. While an effective vaccine is available, present treatment regimens for hepatitis B are costly and often have limiting side effects (25, 60). Only about one-third of patients treated with alpha interferon show a sustained response (28, 36, 60). Nucleoside analogues do not eliminate the virus completely and may select resistant viral variants (59). The development of new treatment strategies remains a major goal but is hindered by the lack of cell lines or a small-animal model infectible with hepatitis B virus that would allow testing.

The causative agent of the disease is HBV, the prototype member of the family *Hepadnaviridae*. These small, DNA-containing viruses replicate through reverse transcription but, in contrast to retroviruses, do not integrate into the host cell genome for replication (49). Infectious virions have a lipoprotein envelope with large (L), medium (M), and small (S) envelope proteins and contain a nucleocapsid. This harbors a small (3 to 3.2 kb), partially double-stranded, relaxed circular DNA (rcDNA) genome with the viral replication enzyme, P protein, covalently attached. After entry into the host cell, the genome is delivered to the nucleus and transformed into covalently closed circular DNA (cccDNA), which serves as a template for transcription. All genomic and subgenomic transcripts are translated into protein. The mRNA for the core and the P protein serves, in addition, as an RNA pregenome. It is copackaged with P protein into newly forming capsids where it is reverse transcribed by the enzyme into DNA (for review, see references 15 and 35).

One characteristic property of the hepadnaviruses is their high species and tissue specificity: HBV infects only humans and humanoid primates or cultured primary hepatocytes of these hosts. Besides virus uptake, viral promoters and enhancers confer hepatocyte specificity during replication (15, 32). In the absence of suitable in vitro or in vivo infection systems for HBV, different experimental systems are in use to study HBV infection. Two related animal viruses are used in their natural hosts: the duck hepatitis B virus (DHBV) (46) and the woodchuck hepatitis B virus (WHV) (43). However, avian and mammalian hepadnaviruses differ in genome structure (46). Even between the closely related mammalian viruses WHV and HBV, differences, e.g., in transcriptional regulation, exist (10). Studies on immunology and pathogenesis of infection are limited by the fact that the natural hosts of these viruses, Peking ducks and woodchucks, are genetically not well defined.

Stable cell lines with integrated HBV genomes, e.g., HepG 2.2.15 cells (47), are commonly used for assessing the action of drugs on HBV replication. HBV-transgenic mice proved to be very useful for immunological studies (17). However, stable cell lines as well as transgenic mice have the disadvantage that, unlike in natural infection, HBV replicates from an integrated genome which cannot be eliminated. In addition, the level of

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Review Article

Drug Therapy

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CALCIUM-ANTAGONIST DRUGS

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DRUGS classified as calcium antagonists or calcium-channel blockers were introduced into clinical medicine in the 1960s and are now among the most frequently prescribed drugs for the treatment of cardiovascular diseases.[1] Although the currently available calcium antagonists are chemically diverse, they share the common property of blocking the transmembrane flow of calcium ions through voltage-gated L-type (slowly inactivating) channels.[2] These drugs have proved effective in patients with hypertension, angina pectoris, and cardiac arrhythmias and may be beneficial in patients with left ventricular diastolic dysfunction, Raynaud's phenomenon, migraine, preterm labor, esophageal spasm, and bipolar disorders.

L-TYPE CALCIUM CHANNELS

All calcium antagonists bind to the α_{1c} subunit of the L-type calcium channel (Fig. 1), which is the main pore-forming unit of the channel. This subunit is associated with a disulfide-linked $\alpha_2\delta$ subunit and an intracellular β subunit. The $\alpha_2\delta$ and β subunits modulate the α_{1c} subunit. The phenylalkylamine (verapamil-like) calcium antagonists bind to transmembrane segment 6 of motif IV (IVS6), the benzothiazepine (diltiazem-like) calcium antagonists bind to the cytoplasmic bridge between motif III (IIIS) and motif IV (IVS), and the dihydropyridine (nifedipine-like) calcium antagonists bind to transmembrane segment 6 of both motif III (IIIS6) and motif IV (IVS6) (Fig. 1).[3]

The L-type calcium channel was first isolated from cardiac muscle and has since been found in vascular smooth muscle (arteriolar and venous), nonvascular smooth muscle (bronchial, gastrointestinal, genitourinary, and uterine), and noncontractile tissues (pancreas, pituitary, adrenal glands, salivary glands, gastric mucosa, white cells, platelets, and lacrimal tissue). Blockade of L-type channels in vascular tissues results in the relaxation of vascular smooth muscle and in cardiac tisssue results in a negative inotropic effect. The ability of these drugs to decrease smooth-muscle and myocardial contractility results in both clinically desirable antihypertensive and antianginal effects and undesirable myocardial depression.

Other calcium channels with electrophysiologic properties have also been identified. These channels, to which the calcium antagonists do not bind, include the N-type channels in neuronal tissue, P-type channels in Purkinje tissues, and T-type (transient potential) channels in cardiac nodal structures and vascular smooth muscle.[4,5]

Regulation of the L-type channels may differ in different types of cells. In cardiac myocytes, these channels are activated by catecholamines and other stimuli that activate adenylyl cyclase or cyclic adenosine monophosphate–dependent protein kinase.[6-8] In contrast, these stimuli activate, inhibit, or have no effect on L-type calcium channels in vascular and visceral smooth-muscle beds, depending on the experimental conditions.[9,10] L-type channels are also activated by the α_1-adrenergic system,[11] angiotensin II,[12] and endothelin.[13] As an in vivo correlate of these findings, calcium antagonists block the responses of vascular smooth muscle to phenylephrine, angiotensin II, and endothelin-1 in humans.[14,15] In addition to hormonal activation by means of signal-transduction pathways, L-type channels may be directly activated at the plasma membrane by guanine nucleotide–binding (G) proteins produced in response to hormone binding to its receptors.

CALCIUM CHANNELS AND CELL GROWTH

L-type (and T-type) calcium channels seem to have a role in cellular growth and proliferation in addition to their role in the acute changes in ion flux associated with changes in membrane potential. Several calcium antagonists, and possibly all, can inhibit the growth and proliferation of vascular smooth muscle and fibroblasts. All classes of calcium antagonists decrease the growth of vascular smooth-muscle cells in vitro and in animals, as measured by decreased uptake of uridine (RNA synthesis) and incorporation of leucine (protein synthesis) at drug concentrations associated with clinical effects.[16,17] Calcium antagonists may also inhibit the synthesis of extracellular-

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